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EDITORIAL

Laboratory Evaluation of A New Molluscicide Application Method

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Abstract

The paper reports an investigation, in the laboratory, of a new method for the application of the molluscicide, Niclosamide, using the concept of slow-release with local materials as matrices. The local matrices tested were the seeds of the mango and the cobs of the maize.
The objective was to reduce the cost of the snail control operations.

1, 5 and 10 seeds and/or cobs were immersed into 4 concentrations (0.6, 1.0, 2.0 and 4.0 ppm) of Niclosamide for different hours (1, 2, 6, 12, 24 and 48 hrs). B. pfeifferi snails were then exposed to the immersed seeds and cobs for different hours (1, 2, 6, 24, 48 and 72 hours).

Different statistical procedures were used to analyze the data. The mean time of mortality of B. pfeifferi snail indicated that the cobs of the maize were a superior matrix for Niclosamide than the seeds of the mango. The LC_{50} and LC_{95} indicated that the combination of 10 cobs immersed for 12 hours in 4.0 ppm was the effective combination to achieve high mortality rates among B. pfiefferi snails after 48 hours exposure. The method proved to be, in the laboratory, cost-effective in terms of the quantity of Niclosamide required to result in a high mortality among the snails.

Introduction

Different methods were used to control schistosomiasis. The measures adopted are either non-specific or specific methods. Non-specific measures are directed towards prevention. The specific tools are directed against the parasite and the intermediate host snails. Different snail control measures were used (Combes & Cheng, 1986). The most widely used method was the chemical control method. Hundreds of chemicals were tested in the laboratory and in the field. At present, the only commercially available molluscicide is Niclosamide (WHO, 2002).

De-Souza (1995) stated that molluscicide application is the most important component in the process of aquatic snail control. Two strategies of snail control are in use in the field, area-wide and focal applications. Area wide application aims at elimination of vectors from all water-courses in an area. Focal application is directed towards the control of the transmission. It is cost-effective and greatly augments chemotherapy (Klumpp & Chu, 1987). Conventionally, molluscicides are applied by hand operated or pressure sprayers, automatic and semi-automatic dispensers, or by aeroplanes or using slow release formulations.

Cardarelli (1974) was the first to suggest the use of slow release molluscicides. The advantages of this method of application are: substantial reduction in cost, greater operational simplicity, less harm to the environment and destruction of free living larval stages of the parasite. The molluscicides that have been tested in slow release systems were copper sulphate, organo-tin, Frescon and Bayluscide (McCullough & Mott, 1983).

Focal chemical control of the intermediate host snails has been recommended and was used in several irrigation schemes in the Sudan (Babiker et al., 1985; BNHP, 1985-1990). The snail control in the irrigation schemes, in the Sudan, is facing many problems, mainly the availability of financial resources to ensure the continuity of the application of the molluscicide and the availability of highly technical trained personnel. There is an urgent need to investigate new methods that are simple, cheap, effective and economically cost-effective.

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The present paper reports the evaluation of a new method of molluscicide application based of the theory of slow-release using maize cobs (Zea mays) and mango seeds (Mangifera indica var. Kitchenar) as a matrix for absorbing and releasing the molluscicide Niclosamide (Bayluscide) under laboratory conditions.

Materials

The maize cobs and the mango seeds:

Maize cobs (cobs) were kindly provided by Agricultural Research Corporation, Wad Medani. Mango seeds (seeds) were obtained from the local market. Cobs and seeds were left to dry completely in the sun for 4 weeks. They were then randomly divided into 3 groups of 1, 5 and 10 cobs and/or seeds. The 5 and 10 cobs and seeds were considered one unit in the experiments.

The concentrations tested:

Four different concentrations of Niclosamide (Bayluscide, Bayer®) 70% w.p. were prepared in a different concentrations, 0.6, 1.0, 2.0, and 4.0 ppm. A solution of 4.0 ppm of Niclosamide was prepared for each experiment and then using the dilution method for the other concentrations.

Preparation of cobs and seeds in Niclosamide:

The 1, 5 and 10 cobs and/or seeds were immersed separately in the 4 different concentrations. A set of 30 containers was prepared for each of the 4 concentrations. The set was divided into 6 groups of 5 containers (repeated 5 times). In each container a known volume of the specified concentration was added according to the number of cobs or seeds to be used. The volume of Niclosamide solution used for immersion of the cobs and seeds were as follows:

Number of materials	1 cob	1 seed	5 cobs	5 seeds	10 cobs	10 seeds
Volume/ml	300	100	700	300	1000	700

The cobs or seeds were then introduced in the container and ensured to be completely immersed by the chemical. The cobs and seeds were immersed for 1, 2, 6, 12, 24 and 48 hours respectively. The cobs and seeds were removed from the molluscicide solution and were left to dry completely in shade. These were designated as the "treated" cobs or seeds. Five replicates of cobs and seeds (1, 5 and 10) were immersed in tap water as a control group.

The Snails:

Biomphalaria snails were collected from Gezira Irrigation Scheme. The snails were washed several times with clean water to remove dirt and mud. They were kept, in the laboratory, in aquaria containing canal water for 3 days until mortality due to stress of transportation had stopped. The surviving snails were screened for patent infection on days 10 and 18 after collection and any snail shedding cercariae was discarded. The snails were maintained in the laboratory until they were needed. They were screened again just before being used in the experiments.

Procedures

A similar set as described above in the preparation of cobs and seeds was used. In each container, 1 litre of water was poured and then 1 treated cob and/or 1 treated seed soaked for 1 hour were put in the containers. 60 B. pfeifferi snails were added to each container. From each container, 10 snails were removed randomly at 1, 2, 6, 24, 48 and 72 hrs. intervals. The removed snails were washed several times with clean water and kept in labelled containers for 24 hrs. before recording the mortality rate. The same experiment was carried out with control groups, using tap water instead of the chemical. The experiment was repeated using 5 cobs and 5 seeds immersed into 5 litres of water. The 10 cobs and 10 seeds were immersed into 10 litres of water. The experiment was repeated 5 times.

Statistical analysis

Statistical tests were used to determine differences in the means of mortality, deviation from the means, standard error and any significant differences between the maize cobs and the mango seeds. All the statistical analysis on the snail mortality were based on the means time of mortality i. e. the average time in which 50% mortality of B. pfeifferi snails occurred.

To determine the mean time of mortality, the release time of the molluscicide from each cob and seed (time of mortality) was classified into 3 class intervals. The range of each class interval was 24 hours. Thus, the mortality rates of the snails exposed for 1, 2, 6, and 24 hours were in class 1, those exposed for 48 hours were in class 2 and those exposed for 72 hours were in class 3 respectively. The mean of 5 readings of snail deaths was multiplied by 100 (mortality rates) and was taken as frequency. The mean time of mortality in relation to the mortality rate and its standard error were calculated for each material used in the 4 concentrations and different times of immersion.

To compare the efficacy of the cobs of the maize and the seeds of the mango, comparison of the means time of mortality per hour was made between cobs and seeds. To measure the significant difference between the two materials, analysis of the variations in the mean time mortality and the standard deviation of B. pfeifferi exposed to cobs and seeds immersed in the different concentrations were calculated.

Confidence intervals were calculated for different probability levels (α = 5%, 1% and 0.1%) to establish the optimum mean time of mortality. Similar analysis was carried out for 5 cobs, 5 seeds, 10 cobs and 10 seeds respectively.

To determine the effective concentration, the means of the mean time of mortality of B. pfeifferi snails exposed to cobs immersed in the 4 concentrations were compared using the t-test. Further analysis was carried out by the Probit procedure to confirm the efficacy of treated cobs according to the snail mortality and to determine the optimum time of immersion and the concentration that can be used.

Results

Mean Time of Mortality According to Time of Immersion:

The mean time of mortality of B. pfeifferi and the confidence intervals of the mean time of the 1 cob/1 seed, 5 cobs/5 seeds and 10 cobs/10 seeds immersed in the 4 concentrations for different hours are shown in Table 1.

There was no obvious significant difference in the mean time of mortality of snails between 1 cob and 1 seed immersed in 3 concentrations of niclosamide (0.6, 1.0 and 2.0 ppm) for different hours (1, 2, 6, 12, 24 and 48 hrs.). However, there was a significant difference in the mean time of the mortality between the 1 cob and the 1 seed immersed for different time intervals in concentration 4.0 ppm of niclosamide ($P < 0.001$). It shows that the mean time that resulted in a high mortality was 24 hrs, that mean time of mortality when the snails were exposed to cobs immersed into 4.0 ppm for 48 hrs. was much longer when compared to the mean time mortality of snails exposed to cobs immersed for 24 hrs.

The confidence intervals at the three levels of confidence showed that the mean time of mortality at 0.6. 1.0 and 2.0 ppm of the cob and the seed overlapped and hence the insignificant difference. At concentration 4.0 ppm, the confidence intervals did not overlap and the mean time for the cob to cause a high mortality was shorter than the mean time for the seed which indicates that the cob at that concentration was more effective than the seed.

There was no obvious significant difference in the mean time of mortality when snails were exposed to 5 cobs and 5 seeds immersed for different hours in 2 of the concentration tested, namely 0.6 and 2.0 ppm. There was a significant difference ($P < 0.02$) in the mean time of mortality of B. pfeifferi snails exposed to 5 cobs and 5 seeds immersed in 1 ppm of niclosamide. However, the confidence intervals of the mean time showed an overlap in their values. The mean time of mortality of the snails exposed to 5 cobs and 5 seeds was highly significantly different ($P < 0.001$) when they were immersed in a concentration of 4.0 ppm of niclosamide.

The confidence intervals of the mean time of mortality of the snails exposed to 5 cobs were shorter than those of the 1 cob immersed in the same concentration. Similar to the 1 cob and 1 seed, there was no

overlap in the confidence limits of the mean time mortality of the snails exposed to 5 cobs and 5 seeds immersed in 4 ppm.

The difference in the mean time of mortality of B. pfeifferi snails, exposed to 10 cobs immersed for different hours, was highly significant ($P < 0.001$) than that of 10 seeds immersed for different hours into concentration 4.0 ppm., but the differences in the mean time of mortality at the other 3 concentrations were not significant.

Again, the mean time of mortality confidence intervals at the three levels of confidence of the 10 cobs and 10 seeds overlapped at concentrations 0.6, 1.0 and 2.0 ppm. Although the confidence intervals of the mean time of mortality of the snails exposed to cobs immersed in 4.0 ppm did not overlap, the time interval was longer than that of 1 or 5 cobs exposed to 4.0 ppm. Similar to 1 cob immersed into 4.0 ppm, the effective concentration was 4.0 ppm, the optimum mean time of exposure that resulted in maximum mortality was 24 hrs., and death of snails exposed to cobs immersed in 4.0 ppm for 48 hrs. took a longer time.

Variation of the Mean Time of Mortality between Cobs and Seeds

The standard deviation was calculated to measure the dispersion of sample observation from the mean value. It was found from the above analysis that the mean time of mortality per hour for B. pfeifferi snails exposed to cobs immersed into 4 ppm was lower than that of the seeds immersed into the same concentration.

It would be expected, therefore, that the standard deviation of the mean time of mortality of the snails exposed to cobs immersed in 4 ppm to be small. This was confirmed in Table 2. The table shows only the mean time of mortality and the standard deviation for the 4 concentrations when the snails were exposed for 24 hours (the exposure time during which maximum mortality rates were observed). These results illustrated that the cobs were a better matrix than the seeds.

It was concluded that cobs were a better matrix than seeds, that concentration 4.0 ppm was more effective than the other concentrations (0.6, 1.0 and 2.0) and that this concentration resulted in a high mortality after 24 hours.

Mean time of mortality

The t-test was used to compare the means of the mean time of mortality of the snails exposed to 1 cob, 5 cobs and 10 cobs immersed in each concentration with the means of the other 3 concentrations. The results of this analysis are shown in Table 3 for the 1 cob, 5 cobs and 10 cobs receptively.

The means of the mean time of mortality of snails exposed to 1 cob immersed into 4.0 ppm were significantly different from the mean time of mortality of snails exposed to 1 cob immersed into 0.6 ($P < 0.002$) and 2.0 ppm ($P < 0.0009$) respectively, but not into 1.0 ppm. However, the difference between the means of mean time of mortality of snails exposed to 1 cob immersed into 1.0 and 4.0 ppm was very large.

The means of the mean time of mortality of the snails exposed to 5 cobs immersed into different concentrations. The results were similar to that of 1 cob, though the mean time of mortality of snails exposed to 0.6 and 2.0 ppm were highly significantly different from those immersed into 4.0 ppm ($P < 0.00004$ & 0.00004 respectively), and there was a significant difference in the means of the mean time of mortality ($P < 0.05$) between 1.0 and 2.0 ppm.

The cross comparison of the means of the mean time of mortality of the snails exposed to 10 cobs immersed into 0.6, 1.0 and 2.0 ppm did not indicate any significant difference in the mortality of snails. However, the comparison of the means of these concentrations with that of 4.0 ppm showed a highly significant difference ($P < 0.002$, 0.00008 & 0.00002 respectively).

Probit Analysis:

The statistical analysis indicated that mortality before 24 hrs. was not significant. The efficacy of the number of cobs immersed for different periods was determined by calculating the LC_{50} and LC_{95} using the Probit analysis.

Calculated Probit mortality of B. pfeifferi snails exposed to 1, 5 and 10 cobs for different periods was found to form a linear relationship with the log concentration. The Chi-square values calculated were

zero, indicating that the data were heterogeneous i. e. the spread of the observed points round the regression line was unequal. Therefore, a heterogeneity factor was used in the calculation of the confidence limits.

LC_{50} and LC_{95} According to Time of Immersion in the Niclosamide:

Table 4 shows the LC_{50} and LC_{95} when the cobs were immersed for different periods in niclosamide with their confidence limits at 95% level, the intercept (slope) of the regression line and the standard error for the intercept.

The calculated LC_{50} and LC_{95} values, after 24 and 48 hrs. of exposure of B. pfeifferi snails to cobs immersed for different hours, increased with increase of immersion time from 1 to 6 hrs. in niclosamide. However, these values decreased for cobs immersed for 12 hrs. before they increased again in those immersed for 24 and 48 hrs. respectively. But when the snails were exposed for 72 hrs., the LC_{50} and LC_{95} continued to decrease with increase of immersion time (1 to 12 hrs.) and they increased with increase in immersion time (24 & 48 hrs. respectively).

The LC_{50} values when B. pfeifferi snails were exposed to cobs for 24, 48 or 72 hrs. ranged between 1.62 and 3.22 ppm respectively. These values were well within the range of the concentrations used in the experiments. The LC_{95} values when the snails were exposed for 24 and 48 hrs. were higher (5.08 & 6.20, 4.37 & 5.07 ppm respectively) than the 4.0 ppm used in the experiments. However, the LC_{95}, when the snails were exposed for 72 hrs. to cobs immersed for 24 and 48 hrs. were well within the range of concentrations used (3.41 & 4.2 ppm respectively).

It was concluded that immersion for 12 hrs. and exposure for 48 hrs. were more cost effective, in terms of the molluscicide required, to achieve high mortality rates.

LC_{50} and LC_{95} According to Number of Cobs:

Table 5 shows the LC_{50} and LC_{95} when the number of cobs, immersed for different periods in niclosamide, was varied with their confidence limits at 95% level, the intercept (slope) of the regression line and the standard error for the intercept.

The calculated LC_{50} and LC_{95} values increased with the increase in the cobs' number. The exception was the LC_{50} and LC_{95} when the snails were exposed to 10 cobs for 72 hrs.

The LC_{50} values when B. pfeifferi snails were exposed to 1, 5 and 10 cobs for 24, 48 or 72 hrs. ranged between 1.93 and 3.03 ppm. These values were well within the range of the concentrations used in the experiments. The LC_{95} range of values when the snails were exposed for 24 hrs., to different numbers of snails, were higher (4.36-5.78 ppm) when compared to the highest concentration used in the experiments (4.0 ppm). However, when the snails were exposed for 48 and 72 hrs., the LC_{95} values were within the range of the concentrations used in the experiments (2.9-4.2 ppm).

Discussion

Worldwide, snail control strategies have relied almost exclusively on chemical control. Molluscicides are applied as granules (Dechiens & Floch, 1970), wettable powder (BNHP, 1982 & 1990; Babiker, 1987), solutions or emulsions (Amin, 1972). Methodology varies from site to site as regards application, dosage and treatment regimen.

Schistosomiasis is endemic in central Sudan, specially in the irrigated schemes. Several trials were carried out to control schistosomiasis in Gezira scheme (Spence, 1924; Sharaf El Din & El Nagar, 1955; El-Nagar, 1958; Amin, 1972, Amin & Fenwick, 1977; BNHP, 1988, 1989 & 1990). The last trial was carried out when the BNHP implemented a comprehensive control strategy in the scheme. The components of the strategy were: health education, provision of water supply, improvement of sanitary condition, focal snail control and chemotherapy. The implementation of this strategy stopped in 1991.

In the irrigation schemes in the Sudan, the snail control programmes failed to stop the transmission of schistosomiasis because the continuity and sustainability of these programmes were interrupted for a

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variety of causes. The lack of proper funding and trained personnel led to incomplete snail control programmes. Controlled-release molluscicides offer a practical and economic method of achieving long-term snail control with the possibility of permanently interrupting the transmission cycle. De-Souza (1995) recommended that for future control strategies it is necessary to use molluscicides formulated for slow release.

The present study is laboratory investigating, a new application method that depends on the controlled release theory, using as a matrix materials available in the local market, in the hope to reduce the cost of the molluscicide applications. The materials tested were the seeds of the mango and the cobs of the maize. Instead of incorporating the chemical in the matrix, the molluscicide will be absorbed and attached to the remaining fibers on the surface of mango seeds and in the pit wells of the maize cobs, when the seeds and cobs are immersed into the molluscicide. The chemicals attached on the surface of the immersed seeds and cobs will then dissolve in water when they are introduced in the snails' environment. The seeds and cobs were immersed into four concentrations of niclosamide 70% wettable powder. However, the method is different from the known slow-release technique in that the release of the chemical from the materials used as matrices is not controlled. When the treated material is introduced in the snail environment, the chemical will dissolve in the water.

The statistical procedures proved that maize cobs are more effective as a matrix for niclosamide under the local conditions. This is because the surface area of the maize cobs are larger than the surface area of the mango seeds. The cobs, having pit wells on the surface, have a wider surface area than the mango seeds. There was no significant difference in the mean time of mortality between the seeds and cobs immersed for different hours in three concentrations of Bayluscide, but the difference was significant at concentration 4.0 ppm. The mortality rates among the three lowest concentrations were less than that at 4.0 ppm. The cobs prove to be superior than the seeds regardless of the number of seeds or cobs used and/or time of immersion in the molluscicide. The mean time of mortality due to exposure to cobs was much less than that of the seeds. The shorter the mean time of mortality, the more efficient the combination (time of immersion, number of material and concentration). This is true for the maize cobs which means that the molluscicide on the cobs retains its molluscicidal activity for a longer period than on the mango seeds. The maize cobs proved to be a better matrix for niclosamide than the mango seeds.

In the present study, the mean time of mortality due to exposure to cobs immersed for 48 hours was higher than that due to exposure to cobs immersed for 24 hours. The reason for the longer mean time of mortality is due to the combined effect of different environmental factors such as pH values of the water, temperature or degradation of the chemical to harmless compounds after 24 hours.

The efficacy of the cobs (LC_{50} and LC_{95}) immersed for different hours in different concentrations of niclosamide increased with the increase of the time of immersion, as well as with the exposure time. This is because as the time of immersion increases more chemical will be accumulated on the surface of the cobs. With a prolonged time of exposure, the chemical dissolves in water slowly and accumulate with time to result in a high mortality among the snails. Immersion for 12 hours and exposure for 48 hours are enough to cause high mortality among B. pfeifferi snails.

Another factor that affected the LC_{50} and LC_{95} is the number of cobs. As the number of cobs and the exposure time increases, the lethal concentrations needed to achieve 50% and 95% mortality decreases. With increasing number of cobs, more of the molluscicide will be accumulated on the surface of the cobs. This will require more time to dissolve in water. It is, therefore, more economical to expose the snails for 48 hours to 10 cobs immersed for 12 hours into 4.0 ppm of niclosamide than any other combination.

It is concluded that, in the laboratory, immersion of 10 cobs for 12 hours into 4.0 ppm of niclosamide and exposure of B. pfeifferi for 48 hours to the immersed cobs will result in the elimination of a substantial percentage of the snails from the environment. The method also proved that very small quantities will be sufficient to cause high mortality rates among the snails. It remains to test the application method in the field. Field evaluation for the application method is urgently needed as well as cost-effective estimation with the conventional application method.

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Table 1: Mean Time of Mortality, Significance and Confidence Intervals of B. pfeifferi Exposed to Different Concentrations, Using Maize Cobs and Mango Seeds

Material	Con (ppm)	Mean Time of Mortality (hr)	Sign.	Confi Inter 95%	Confi Inter 99%	Confi Inter 99.9%
1 Cob	0.6	27.16	N. S.	24.86 - 29.45	24.13 - 30.15	23.18 - 31.07
1 Seed		28.97		26.85 - 31.09	26.14 - 31.98	25.25 - 32.57
1 Cob	1.0	23.20	N. S.	21.56 - 24.85	21.02 - 25.40	20.34 - 26.08
1 Seed		20.02		19.28 - 20.76	19.03 - 21.00	18.72 - 21.31
1 Cob	2.0	26.80	N. S.	23.90 - 29.70	23.01 - 30.01	22.01 - 31.18
1 Seed		29.75		27.59 - 31.91	26.81 - 33.03	25.85 - 33.82
1 Cob	4.0	13.51	***	13.27 - 13.76	13.18 - 13.84	13.03 - 13.94
1 Seed		26.62		24.58 - 28.66	23.90 - 29.57	23.07 - 30.17
5 Cobs	0.6	27.73	N. S.	25.13 - 30.34	24.08 - 31.39	22.95 - 32.53
5 Seeds		29.92		25.27 - 32.58	24.55 - 30.31	23.66 - 31.20
5 Cobs	1.0	20.36	*	18.35 - 22.38	17.68 - 23.05	16.85 - 23.88
5 Seeds		23.16		22.02 - 24.31	21.65 - 24.69	21.18 - 25.16
5 Cobs	2.0	27.80	N. S.	25.49 - 30.12	24.58 - 34.06	23.59 - 32.03
5 Seeds		31.04		29.03 - 33.05	28.25 - 33.83	27.52 -34.50
5 Cobs	4.0	12.00	***	12.00	12.00	12.00
5 Seeds		27.26		25.32 - 29.21	24.68 - 29.86	23.87 - 30.62
10 Cobs	0.6	30.36	N. S.	26.90 - 33.83	25.84 - 33.35	24.67 - 34.57
10 Seeds		27.17		25.46 - 28.88	24.73 - 29.44	23.74 - 30.50
10 Cobs	1.0	29.93	N. S.	27.46 - 32.40	26.63 - 33.23	25.66 - 34.20
10 Seeds		30.39		28.26 - 32.52	27.36 - 33.26	26.44 - 34.18
10 Cobs	2.0	27.96	N. S.	24.95 - 30.97	23.95 - 31.96	22.76 - 33.20
10 Seeds		30.30		27.91 - 32.70	27.10 - 33.58	26.09 - 34.58
10 Cobs	4.0	19.04	***	17.14 - 20.95	16.31 - 21.62	15.49 - 22.44
10 Seeds		30.19		28.24 - 32.14	27.35 - 33.07	26.46 - 33.95

* P. value < 0.02
*** P. value < 0.001

Table 2 : Mean Time of Mortality and Their Standard Deviations of B. Pfeifferi Exposed Cobs and Seeds Immersed into 4 Concentrations of Niclosamide for 24 Hours

Concent.	Mean Time 1 Cob (hr)	S. D. (Cob)	Mean Time 1 Seed (hr)	S. D. (Seed)
0.6	22.86	8.14	20.00	7.77
1.0	21.00	10.73	25.71	8.94
2.0	25.07	5.93	28.91	6.39
4.0	12.00	5.91	00.00	8.25
Concent.	Mean Time 5 Cobs (hr)	S. D. (Cob)	Mean Time 5 Seeds (hr)	S. D. (Seed)
0.6	29.33	8.22	31.20	9.60
1.0	00.00	0.00	12.00	0.00
2.0	31.58	6.98	32.72	7.40
4.0	12.00	0.00	12.00	0.00
Concent.	Mean Time 10 Cobs (hr)	S. D. (Cob)	Mean Time 10 Seeds (hr)	S. D. (Seed)
0.6	30.32	8.14	30.78	7.77
1.0	24.00	10.73	32.00	8.94
2.0	33.65	5.93	33.33	6.39
4.0	19.06	5.91	32.00	8.25

Table 3 The Difference Between Means of the Mean Time of Mortality, Significance and Confidence Level Between the Different Concentration for 1 Cob

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Concentration (ppm)	Difference between means	Sig.	Confidence Level
0.6 vs. 1.0	3.92	N. S.	0.50600
0.6 vs. 2.0	1.96	N. S.	0.37900
1.0 vs. 2.0	1.96	N. S.	0.87150
0.6 vs. 4.0	13.62	***	0.99780
1.0 vs. 4.0	9.70	N. S.	0.94340
2.0 vs. 4.0	11.66	***	0.99903
Concentration (ppm)	Difference between means	Sig.	Confidence Level
0.6 vs. 1.0	7.37	N. S.	0.87520
0.6 vs. 2.0	2.45	N. S.	0.88900
1.0 vs. 2.0	9.82	*	0.95000
0.6 vs. 4.0	15.74	****	0.99996
1.0 vs. 4.0	8.37	N. S.	0.91000
2.0 vs. 4.0	18.19	****	0.99996
Concentration (ppm)	Difference between means	Sig.	Confidence Level
0.6 vs. 1.0	0.31	N. S.	0.56930
0.6 vs. 2.0	1.24	N. S.	0.85140
1.0 vs. 2.0	0.93	N. S.	0.67140
0.6 vs. 4.0	10.80	***	0.99880
1.0 vs. 4.0	11.11	****	0.99920
2.0 vs. 4.0	12.04	****	0.99998

*P. value < 0.02 *** P. value < 0.001 **** P. value < 0.0001

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Table 4 : LC_{50} and LC_{95} of Cobs Immersed for Different Hours in Four Concentrations of Niclosamide

LC_{50} and LC_{95} after 24 hrs. exposure to cobs immersed for different periods in niclosamide

Imm Time	LC_{50}	Con. Inter. 95%	LC_{95}	Con. Inter. 95%	Intercept	S.E.
1	2.41433	2.1135 - 2.7571	4.6487	3.9986 - 5.5635	-2.21288	.04918
2	2.50876	2.0274 - 2.8555	4.8305	4.1673 - 5.7923	-2.30920	.04988
6	2.98861	2.6213 - 3.3993	5.7548	4.9758 - 6.8380	-2.74860	.05619
12	2.23867	1.9561 - 2.5519	4.3105	3.7289 - 5.1107	-2.02323	.04922
24	2.63944	2.3128 - 3.0120	5.0821	4.3728 - 6.0823	-2.43668	.05139
48	3.22213	2.8130 - 3.6974	6.2041	5.3036 - 7.4868	-2.93748	.05139

Statistical Analysis
Regression Coefficient = 5.780820 S.E. = 0.090120
χ^2 = 8194.283 P = 0.000

LC_{50} and LC_{95} after 48 hrs. exposure to cobs immersed for different periods in niclosamide

Imm Time	LC_{50}	Con. Inter. 95%	LC_{95}	Con. Inter. 95%	Intercept	S.E.
1	2.32278	2.0856 - 2.5868	4.3752	3.8716 - 5.0304	-2.18927	.04836
2	2.08913	1.8735 - 2.3279	3.3951	3.4841 - 4.5188	-1.91386	.04650
6	2.01291	1.8897 - 2.3350	3.9610	3.5259 - 4.5176	-1.93094	.04726
12	1.78674	1.5962 - 1.9947	3.3655	2.9839 - 3.8522	-1.57700	.04508
24	2.31997	2.0762 - 2.5907	4.3699	3.8617 - 5.0283	-2.18612	.04960
48	2.69055	2.4079 - 3.0072	5.0679	4.4693 - 5.8487	-2.57108	.05282

Statistical Analysis
Regression coefficient = 5.981470 S.E. = 0.189130
χ^2 = 5517.041 P = 0.000

LC_{50} and LC_{95} after 72 hrs. exposure to cobs immersed for different periods in niclosamide

Imm Time	LC_{50}	Con. Inter. 95%	LC_{95}	Con. Inter. 95%	Intercept	S.E.
1	2.19136	1.9488 - 2.4608	3.8249	3.3636 - 4.4459	-2.31662	.04836
2	1.93429	1.7130 - 2.1772	3.3762	2.9692 - 3.9167	-1.98951	.04650
6	1.89987	1.6817 - 2.1382	3.3161	2.9186 - 3.8419	-1.89513	.04726
12	1.61913	1.4339 - 1.8208	2.8261	2.4899 - 3.2698	-1.42298	.04508
24	1.95553	1.7450 - 2.1914	3.4133	3.0023 - 3.9717	-1.98040	.04960
48	2.30757	2.0455 - 2.6021	4.0278	3.5260 - 4.7074	-2.46920	.05282

Statistical Analysis
Regression coefficient = 6.799310 S.E. = 0.119160
χ^2 = 6047.214 P = 0.000

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Table 5 : LC_{50} and LC_{95} According to the Number of Cobs Immersed for Different Periods in Four Concentrations of Niclosamide

LC_{50} and LC_{95} after 24 hrs. exposure to different numbers of cobs immersed for different periods						
Cobs No.	LC_{50}	Con. Inter. 95%	LC_{95}	Con. Inter. 95%	Intercept	S.E.
1	2.2895	2.0800 - 2.5147	4.3651	3.8799 - 5.0466	-2.1147	.04113
5	2.7630	2.5089 - 3.0427	5.2679	4.6582 - 6.1346	-2.5906	.04591
10	3.0333	2.7458 - 3.3505	5.7831	5.7831 - 5.1033	-2.8285	.04940
Statistical Analysis Regression Coefficient = 5.86930 S.E. = 0.08794 χ^2 = 9099.506 P > 0.000						
LC_{50} and LC_{95} after 48 hrs. exposure to different numbers of cobs immersed for different periods						
Cobs No.	LC_{50}	Con. Inter. 95%	LC_{95}	Con. Inter. 95%	Intercept	S.E.
1	2.0937	1.9620 - 2.2330	3.30487	3.0499 - 3.6394	-2.6629	.0552
5	2.1660	2.0314 - 2.3104	3.41896	3.1496 - 3.7754	-2.7900	.0555
10	2.6417	2.4686 - 2.8222	4.16988	3.8509 - 4.5836	-3.5004	.0699
Statistical Analysis Regression coefficient = 8.29722 S.E. = 0.14239 χ^2 = 5604.687 P > 0.000						
LC_{50} and LC_{95} after 72 hrs. exposure to different numbers of cobs immersed for different periods						
Cobs No.	LC_{50}	Con. Inter. 95%	LC_{95}	Con. Inter. 95%	Intercept	S.E.
1	2.0543	1.9160 - 2.2038	3.0859	2.7961 - 3.5629	-2.9104	.08739
5	2.1881	2.0363 - 2.3590	3.2899	2.9622 - 3.2588	-3.1655	.09199
10	1.9353	1.7918 - 2.0681	2.9014	2.6524 - 3.2962	-2.6612	.08793
Statistical Analysis Regression coefficient = 9.30813 S.E. = 0.25960 χ^2 = 6127.734 P > 0.000						

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